CCXX. THE CHEMICAL COMPOSITION OF TEETH.

IV. THE CALCIUM, MAGNESIUM AND PHOSPHORUS CONTENTS OF THE TEETH OF DIFFERENT ANIMALS. A BRIEF CONSIDERATION OF THE MECHANISM OF CALCIFICATION.

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BECAUSE of the significance usually attached to the determination of ash, Ca and Ca/P ratio in bones and teeth in relation to diet and disease, it is important to give due consideration to the interrelationship of the Ca and Mg contents and the ash values. Orent *et al.* [1934] have studied the question of Mg deficiency in rats and shown how the Mg content of the bones varies with age in control animals and in animals on diets deficient in Mg. These differences in Mg content caused related inverse changes in Ca content and resulting changes in ash content (1 g. Mg $\equiv$ 1·648 g. Ca).

Having determined the Ca/P ratio in numerous samples of human teeth, where the values fell between 1·92 (lowest dentine) and 2·153 (highest enamel), it was surprising to find values as low as 1·7 and 1·8 in rats' incisor teeth, although rats' and rabbits' bones gave the expected ratios of 2·13 to 2·15, confirming the generally accepted values. This led to the investigation of the amount of Mg present in dental tissues from various sources and to the correlation of the results with the Ca and P contents.

Many of the usual phosphate precipitation methods for Mg determination were tried but finally rejected in favour of the hydroxyquinoline method of Greenberg & Mackey [1932]. This method adapted to determinations in solutions of dental material proved very satisfactory with an error of about $\pm 0{\cdot}5\,\%$. Table I gives the results of Ca, Mg and P determinations on the ash of various dental tissues. The Ca/P ratios were calculated. The Mg values were converted into their equivalent amounts of Ca, which values were then added to the corresponding actual Ca contents to give "Total Ca". Using these total Ca values, "Corrected Ca/P" ratios were calculated.

It was decided to compare the values of the rodents' whole teeth with the dentine of horse, dog and human, rather than with the enamel. The differences in composition between dentine and enamel have been dealt with in a previous publication [Bowes & Murray, 1935]. Enamel has on an average 0·4 % Mg.

In the consideration of the results, certain points relating to the theories of calcification, which have been dealt with fully by Robison [1932], have been borne in mind. The generally accepted view is that calcification takes place in calcifying tissues from solutions rendered saturated or supersaturated when the product ($Ca^{++} \times PO_4^{\equiv}$) exceeds the solubility product. The material deposited, "the bone salt", is an insoluble phosphate of the apatite series $3Ca_3(PO_4)_2 . CaX_2$.

Table I.

Material	% in ash Ca	Mg	P	Ca/P	Mg expressed as Ca	"Total" Ca	Ca/P "Corrected"
Rabbit incisors	35·02	2·467	19·88	1·761	4·061	39·08	1·965
Rabbit molars	35·76	1·45	20·15	1·775	2·39	38·15	1·893
Hare incisors	35·33	2·304	20·16	1·753	3·797	39·13	1·941
Hare molars	36·41	1·788	19·25	1·891	2·947	39·36	2·045
Guinea-pig incisors	35·80	1·745	19·56	1·831	2·876	38·68	1·978
Guinea-pig molars	35·01	1·932	20·04	1·748	3·185	38·20	1·903
Rat 12 weeks incisors	36·20	2·243	19·68	1·840	3·697	39·90	2·027
Rat 15 weeks incisors	34·82	2·363	19·62	1·774	3·895	38·72	1·973
Rat 1 year ditto	38·85	1·741	20·01	1·917	2·869	41·72	2·085
Horse dentine	37·12	1·855	19·23	1·931	3·058	40·18	2·101
Dog dentine	37·61	1·545	19·27	1·952	2·547	40·16	2·095
Elephant dentine (tusk)	32·91	4·055	19·64	1·675	6·681	39·60	2·016
Human dentine* sound, permanent, newly erupted, premolars	39·83	1·175	19·04	2·091	1·936	41·77	2·193
Human dentine sound, permanent, old (Indian)	37·81	1·310	18·46	2·048	2·165	39·97	2·165
Human dentine sound, temporary	37·64	1·391	18·18	2·071	2·293	39·93	2·189
*Corresponding enamel	39·98	0·424	18·42	2·171	0·70	40·68	2·206

Robison [1932] considered that in all probability the other inorganic constituents of bones and teeth can be incorporated into this complex molecule, equivalent amounts of other bases replacing the Ca and various groups CO_3, (OH_2), Cl_2 and F_2 representing the X_2. The Ca/P ratio of such a compound is 2·153. Replacement of one Ca by Mg would give a ratio of 1·935, the same as that of tricalcium phosphate $Ca_3(PO_4)_2$. If Mg does replace Ca in the apatite of bones and teeth, then having calculated the amount of Ca equivalent to the Mg, the Ca/P ratio should approximate to that of apatite. Orent *et al.* [1934] have shown that Mg and Ca do replace one another in rats' bones.

The results in Table I show that the human dentine contained the least Mg and gave the highest Ca/P ratio both actual and corrected. The corrected value was close to that of apatite. Next in order came the horse and dog dentine with corrected ratios differing a little from that of apatite. The P values of human, dog and horse dentine differed very little from one another. Taken as a whole the results from these dentines agreed reasonably with the theory that the calcified substance is largely apatite. Human enamel corresponds very closely to apatite. The elephant tusk dentine (ivory) had a very high Mg content and hence a correspondingly low Ca. The actual Ca/P ratio in this would be a very misleading figure if not taken in conjunction with the corrected ratios. In passing to the rodents' teeth the results showed wide differences from those of the dentines. These teeth in general had a much greater Mg content and consequently a lower Ca content. Toverud [1923] found only 0·63 % Mg in rats' incisors, but Watchorn (private communication) found 1·6 % Mg in 4½-month-old rats on a diet containing 0·045 % Mg. In the present work values are high, no doubt because the diet was unintentionally but not abnormally high in Mg, i.e. 0·15 %. The proportion of Ca:Mg in these different materials shows that there are variations in the degree of replacement. In some cases (human and dog dentine) the molar relation Ca:Mg was approximately 18:1, in rodents' incisors 9:1, whilst in the elephant ivory it was 5:1 approximately. The difference in Mg content cannot altogether be accounted for by variations in Mg content of the blood sera. Human, dog, rabbit and rat sera contain respectively 2·2 [Greenberg & Mackey, 1932], 2·4 [Greenberg & Mackey, 1932], 2·6 [Brookfield, 1933], and 5·0 [Watchorn, 1933], all stated as mg. Mg/100 ml. The P values in rats' teeth were also significantly higher. The resulting Ca/P ratios were also much too low to satisfy $3Ca_3(PO_4)_2.MgX_2$. It is probable that Mg replaces even more of the Ca. A consideration of the "total" Ca and P values in comparison with the corresponding values in human dentine, etc. suggests that some other P compound, less basic than tricalcium phosphate, is present in rodents' teeth or that they contain considerable quantities of other bases not estimated. This still does not explain why the P content of the rodents' teeth was so much greater. X-ray analysis of rats' teeth and elephant tusk is being carried out in conjunction with this work and should help to settle the question of constitution.

A notable point is the difference in composition of the incisor and molar teeth of the same animal. The high Mg and low Ca/P of rats' incisors prompted this investigation on rabbits' and guinea-pigs' teeth. The rabbit and hare incisors corresponded reasonably well with rats' incisors. It was surprising to find much lower Mg values in guinea-pigs, but this was in agreement with the results of Toverud [1923] who found 1·74 % Mg. The difference between the incisors and molars in some cases is very marked and it does not seem possible to explain the differences by reason of the different proportions of enamel and dentine in the different teeth. The variations in Mg content of the rats' incisors at different ages agreed with the observations of Orent *et al.* [1934].

It would seem from the differences in composition of incisor and molar teeth of certain rodents and from the fact that the enamel and dentine which are laid down, presumably at the same time, in human teeth, can show distinct differences in Ca:Mg:P proportions, that the physico-chemical conditions which according to the supersaturation precipitation theory govern the deposition must be subject to local variations and not be determined by the blood system only. It may be noted in passing that though the Ca contents of different animals' blood do not differ very much, the P contents show big differences; for example pigs' blood contains much more inorganic P and ester-phosphorus than the blood of most other animals [Kay, 1928].

The fact that the proportion of Ca:Mg varies with age has also to be accounted for. Day *et al.* [1935] have dealt with this variation of Mg content of bone. It is possible that these age changes could be correlated with the recognized differences in blood composition at different ages [Watchorn, 1933], but it is not so easy to correlate the differences between incisor and molar teeth and bone taken at the same time, or the difference between enamel and dentine in human teeth. In the ash of human enamel the Ca/P is 2·15 and Mg 0·46 %, in the ash of dentine the Ca/P is 2·05 and Mg 1·2 %. The fact that big differences in mineral content can occur in material deposited in different regions of the body at the same time promotes the suggestion that all calcifications are the result of specific cell activity rather than merely precipitation governed by solubility products. Logan [1935] observed similar differences in composition of the inorganic part of calcified tissues and is of the opinion that these differences must be taken into account in any consideration of the means by which calcification takes place. There is evidence that the deposition of salts in bone, designated by Robison *et al.* [1930] the "inorganic" or second process in calcification (the hydrolysis of phosphoric esters by phosphatase apparently precedes this), is not a purely passive physico-chemical process because of the fact that *in vitro* it is inhibited by various means, partially by 0·001 M KCN, profoundly by 0·00001 M NaF [Robison & Rosenheim, 1934]. It is usual to associate such inhibitions with the cell activity. The last mentioned authors brought forward strong evidence that the second mechanism was also enzymic. For several reasons then calcification might be considered similar to other active and specific cell processes. The ameloblasts, odontoblasts and osteoblasts each, by the various enzymic processes, are responsible for laying down in an organic matrix complex phosphates of calcium and magnesium. The complexes are not necessarily universally identical but are affected by local conditions as well as by conditions in the blood. The organic composition of the matrix may be important in connexion with the variations in composition of the inorganic substance laid down therein. Harris [1932] found glycogen in developing bone. With the exception of liver cells in which glycogen represents a stored secretion, glycogen is usually regarded as a ready store of energy which is made available for cellular activity by formation of various esters of phosphoric acid. The glycogen of developing bone could play a similar role in the active cell processes. That glycogen metabolism may play a part in calcification is indicated by the fact that sodium iodoacetate in very low concentration inhibits the calcifying process *in vitro* [Robison and Rosenheim, 1934] and *in vivo* [Laszt & Verzár, 1935].

The theory built up by Robison from his extensive work on the calcifying mechanism has emphasized the sensitive character of the second mechanism. The results put forward here suggest that the calcification process is highly specific and that the differences in composition of the inorganic substance laid down in calcifying tissues are to be explained on the basis of this specificity.

Summary.

1. A quantitative study of the Ca, P and Mg contents of the ash of teeth of different animals has been made. Marked differences were found in the relative amounts of these elements present.

2. Rodents' teeth generally contained more Mg, more P, but less Ca than dog and human teeth. Rodents' incisors and molars were different in composition.

3. Ca/P ratios varied between 1·75 (rats' incisors) and 2·09 (human dentine). This variation was due chiefly to differences in the Ca content resulting from the differences in Mg content. Ca/P ratios give no idea of the type of phosphate present. "Corrected" Ca/P ratios (see text) differed less.

4. The suggestion is made that the wide differences in composition of the mineral constituents found in teeth of different animals, in different types of teeth of the same animal, in bones and teeth of the same animal and in different tissues in the same tooth, support the view that the deposition process of calcification is an active and specific cell process, and not merely a precipitation from saturated or supersaturated solutions of a salt of constant composition dependent on the ionic composition of the blood plasma.

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